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THE EFFECTS UPON THE BRONCHIAL MUSCULATURE OF ALTERING THE OXYGEN AND CARBON DIOXIDE TENSIONS OF THE BLOOD PERFUSING THE BRAIN

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Roy & Brown (1885) showed that, in dogs, asphyxia could cause bronchoconstriction or bronchodilatation, but they did not localize the origin of these effects. Evidence that changes in gaseous composition of the blood influences the bronchial musculature by a central mechanism was first obtained by Einthoven (1892). He found that, in dogs, inhalation of high concentrations of carbon dioxide caused bronchoconstriction which was abolished by section of the vagus nerves. This was confirmed, in cats, by Dixon & Brodie (1903), although in one experiment they found that section of the vagus nerve on the same side as the lung lobe from which they were recording bronchomotor responses did not entirely abolish the bronchoconstrictor response to inhalation of carbon dioxide. They concluded that there might be both a central and peripheral effect. In the light of more recent work, it is possible that the lung lobe still received a vagal innervation from the contralateral side, since it has been shown that there is a considerable crossed vagal innervation of the lungs in the dog (Braeucker, 1926; I. de B. Daly & Hebb, 1942), and cat (Dixon & Ransom, 1912; Daly & Mount, 1951). In one experiment, Einthoven (1892) found that inhalation of nitrogen caused bronchoconstriction.

Using the technique of perfusing the isolated head connected with its trunk only by the cervical vagosympathetic nerves, Houssay & Cruciani (1929) found that central anaemia caused bronchoconstriction in the trunk of the recipient. This was confirmed by Daly & Schweitzer (1952) using a similar technique, but a different method of recording bronchomotor responses. Intracarotid injections of blood containing a low oxygen content but a normal carbon

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dioxide content also causes bronchoconstriction due to a central mechanism (Daly & Schweitzer, 1952).

These findings strongly suggest that the bronchial musculature is under a central control, although the results of some of the earlier workers do not exclude the participation of reflexes from the carotid sinus region.

In the present investigation, we have perfused the brain of the dog *in situ* to study the bronchomotor effects of altering the gas tensions of the blood passing through it. The advantage of the technique used is that the oxygen and carbon dioxide tensions can be altered independently. No attempt has been made to localize the part of the brain responsible for bringing about the observed responses, but the evidence which will be presented leaves no doubt that the effects are central in origin. It has been shown that they are all mediated through the vagus nerves, and it is therefore generally assumed that such effects are due to an action on the vagus centre in the medulla. Some of our results have already been reported (Daly, Lambertsen & Schweitzer, 1952).

METHODS

Dogs, varying in weight from 7·5 to 15·1 kg, were anaesthetized with either chloralose (0·1 g/kg body weight, intravenously), sometimes preceded by morphia (2 mg/kg body weight, subcutaneously), or pentobarbitone sodium (Nembutal) (45 mg/kg body weight, intraperitoneally).

The animals were artificially ventilated by means of a Starling 'Ideal' pump and the chest was opened in the mid-sternal line. The expiratory side of the pump was connected by a tube, the end of which was immersed 1–3 cm under water thus preventing complete collapse of the lungs during expiration (Fig. 1, a_1). The lungs were ventilated with 100 % oxygen throughout the experiment. For this purpose the input side of the pump was connected to a cylinder of O_2; the flow was regulated to keep a breathing bag expanded at a pressure of 2–3 mm water maintained by the water valve, *b*. The inspired air was warmed by passing the tubing through a water-bath maintained at 37° C.

The brain was perfused by means of a Dale & Schuster (1928) pump, using the animal as the blood 'reservoir' to supply the pump (I. de B. Daly & Duke, 1948; Gaddum, Peart & Vogt, 1949). A cannula was inserted into each auricle through the auricular appendix and connected to the three-way taps T_1 and T_2 on the input side of the pump chamber; the output side was connected to cannulae, pointing rostrally, inserted into either the common carotid or the vertebral arteries near their origin from the subclavian arteries. After filling the pump with blood, perfusion was begun from the left auricle. The blood, before entering the cannulae, passed through a jacketed glass coil (c_1) maintained at 37° C by water circulated from the thermostatically controlled water-bath. The temperature of the inflowing blood was measured by a thermometer. The perfusion pressure was measured by means of a mercury manometer. By turning taps T_1 and T_2 so as to put the input side of the pump in communication with the right auricle, mixed venous blood could be perfused through the brain. The perfused blood was returned to the right heart through the normal venous channels.

Certain precautions were taken to ensure that the perfusing blood was diluted as little as possible, on its passage through the brain, with blood from other sources. When perfusion was carried out through the vertebral arteries, the carotid blood was prevented from reaching the brain by ligature of the internal and external carotids, occipital and ascending pharyngeal arteries on both sides. Likewise, when perfusion was carried out through the common carotid arteries, both vertebral and external carotid arteries were ligatured. The considerable spinal contribution to the blood supply of the brain of dogs (Hill, 1896; Evans & Samaan, 1936;

Chungcharoen, Daly, Neil & Schweitzer, 1952) was eliminated as far as possible by adjusting the output of the pump so that the perfusion pressure was 30–80 mm Hg higher than the systemic blood pressure. To prevent carotid sinus and carotid body reflexes, both carotid sinus nerves were cut in nearly all experiments. The blood was rendered incoagulable by heparin ('Liquemin', Roche Products Ltd.) (7·6 mg/kg).

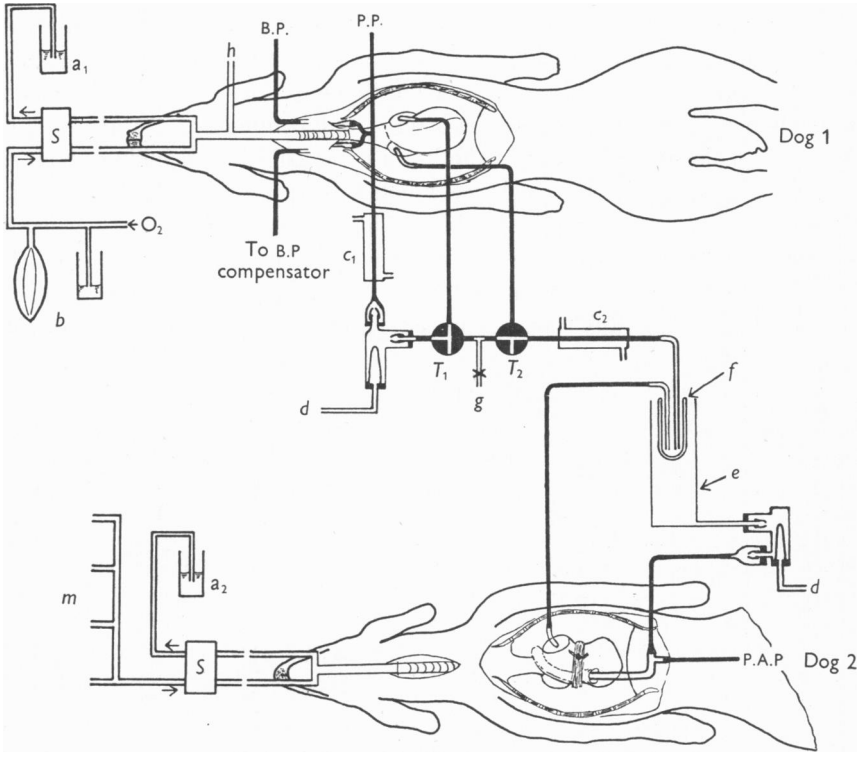

Fig. 1. Method of perfusing the brain. For details see text. a_1, a_2, water valves allowing slight positive expiratory pressure; *b*, breathing bag and water trap for delivery of 100 % O_2 to input of Starling 'Ideal' pump; c_1, c_2, warming coils; *d*, connexion to Dale-Schuster pump; *e*, outer blood reservoir; *f*, inner blood reservoir; *g*, side tube; *h*, connexion to respiratory constant pressure device and overflow recorder for measuring bronchomotor responses (method of Konzett & Rössler, 1940); *m*, manifold for Douglas bags; *S*, Starling 'Ideal' pump; T_1, T_2, three-way glass taps; B.P., systematic blood pressure, and P.P., vertebral perfusion pressure, measured with mercury manometers; P.A.P., pulmonary arterial pressure measured with saline manometer. Through taps, T_1 and T_2, arterial blood perfusing the brain from the left auricle may be changed for mixed venous blood from the right auricle or for blood equilibrated with various gas mixtures in the isolated perfused lungs.

In some experiments the oxygen and carbon dioxide tensions of the blood perfusing the brain were altered independently using the isolated perfused lungs of a second dog as an equilibrator.

These dogs varied in weight from 6·5 to 13 kg and were bled to death from the femoral artery under local anaesthesia and either pentobarbitone sodium (Nembutal) (15 mg/kg body weight, intraperitoneally) or morphia (2 mg/kg body weight, subcutaneously). Heparin (7·6 mg/kg) was injected into the femoral vein before bleeding. After ligation of the superior and inferior venae

cavae and the vena azygos, the lungs were perfused *in situ* at a constant blood volume inflow, through the pulmonary artery. Blood from the left auricle was returned to the bottom of the water jacketed inner reservoir *f* (capacity 75 ml.), spilt over into the large reservoir *e*, and was then returned to the pulmonary pump (Fig. 1). A second tube from the bottom of the inner reservoir conveyed blood to the input side of the brain perfusion pump through taps T_2 and T_1. The pulmonary arterial pressure was measured with a vertical saline manometer. Lung blood flows were 150–450 ml./min at 20–25 cm saline pressure. Water was circulated through the warming coil, c_2, and through the inner reservoir (*f*) from the water-bath maintained at 37° C. The large reservoir and pulmonary pump were also immersed in the bath.

The isolated lungs were ventilated by means of a Starling 'Ideal' pump; a positive pressure during the expiratory phase of respiration of 2–3 cm water was maintained by the water valve a_2. The inspiratory side of the pump was connected with a manifold *m* to which were attached Douglas bags containing various mixtures of oxygen, carbon dioxide and nitrogen. Ten minutes were allowed for equilibration of the blood. Before the brain perfusion was switched over to blood from the inner reservoir, the blood remaining in the tube between tap T_2 and the inner reservoir from the previous test was replaced with newly equilibrated blood through tube *g*. The blood in the tube of tap T_1 was displaced by turning tap T_1 to communicate with the inner reservoir for a few seconds. After an interval of 1 min (the time required for that blood to have passed through the brain), the next test could be carried out. Thus, in these experiments, arterial blood perfusing the brain from the left auricle (dog 1) could be changed to mixed venous blood from the right auricle or to blood from the perfused lungs which had been equilibrated with the desired gas mixture.

Systemic blood pressure was recorded from the left common carotid or the femoral artery by means of a mercury manometer. The right common carotid artery was connected to a blood-pressure compensator similar to that described by Roberts (1921), but modified according to Daly & Schweitzer (1952). Such a device was necessary when perfusing the brain with blood from the inner reservoir; the blood entering the dog at a rate of 85–120 ml./min did not then cause a rise in blood pressure and hence reflex bronchomotor effects through the aortic nerves (Daly & Schweitzer, 1951). The compensator was emptied at intervals and the blood replaced in the large reservoir (*e*).

In these experiments, a third dog was used to provide extra blood for the reservoirs and blood-pressure compensator. It was bled under the same conditions as the dog used for the preparation of the isolated lungs.

In all experiments, the bronchomotor responses were measured by the method of Konzett & Rössler (1940); the constant positive inflationary pressure varied from 8 to 13 cm water in different experiments. The ventilation overflow volume was recorded by means of a piston recorder. Heart rate was measured by the method described by Daly & Schweitzer (1950), using a Gaddum drop timer (Gaddum & Kwiatkowski, 1938).

The advantages of passing shed blood through the lungs before being perfused through an organ for decreasing the concentration of vasotonins have been stressed previously (see Hebb & Linzell, 1951). The formation of vasotonins can be further minimized by preventing blood from becoming stagnant in reservoirs. This led us to incorporate the small reservoir in the isolated lung-perfusion circuit so that the blood in it is continually circulating and being renewed. It has the added advantage that the time needed to equilibrate blood with gas mixtures is less than if only one large reservoir had been used.

Blood analysis. During the course of the experiments, blood samples were taken from the left and right auricular tubing (dog 1) and from the tubing connecting tap T_2 with the inner reservoir during the various tests. They were collected in 5 ml. glass syringes and stored in crushed ice pending analysis.

The carbon dioxide and oxygen contents of the blood were determined manometrically by the method of Van Slyke & Neill (1924). In most instances only a single analysis was performed on a blood sample, this being considered adequate for our purposes. Determinations were completed

within 6 hr of withdrawal of the blood specimens. Single determinations of the haemoglobin concentration were made photometrically as cyanmethaemoglobin (Drabkin & Austin, 1935) with a Hilger Spekker photoelectric absorptiometer using, as a reference standard, a cyanmethaemoglobin solution prepared from blood, the oxygen capacity of which had been determined by a manometric method of Van Slyke & Neill (1924). Oxygen capacity was calculated from the haemoglobin concentration on the assumption that 1 g haemoglobin combines with 1·34 ml. oxygen.

Estimations of blood pH were performed anaerobically in duplicate at room temperature (18–21° C) with a MacInnes-Belcher glass electrode and a Marconi type TF 717 pH meter. The apparatus was standardized before each determination by means of phosphate buffers of pH 7·20 and 7·40 at 20° C. Measurements of blood pH at ambient temperatures were completed within 4 hr after sampling and were converted to the corresponding values of 37° C by the temperature coefficient of Rosenthal (1948).

Carbon dioxide tension was estimated from the observed values for carbon dioxide content, pH, haemoglobin content and percentage oxygen saturation of whole blood by means of the nomograms of Van Slyke, Sendroy & Lui (1932) and Van Slyke & Sendroy (1928).

Cardiac output determinations. In the course of a few experiments, cardiac output determinations were made according to the direct Fick principle before and after opening the chest in the mid-sternal line. Arterial blood samples were taken from the femoral artery and mixed venous blood samples from a cannula in the right auricle. In the determinations carried out before the chest was opened, a glass cannula was inserted into the right auricle through the right external jugular vein; after opening the chest, the samples were drawn directly from the cannula inserted into the right auricular appendix. Simultaneously, oxygen consumption was measured with a small bell spirometer in a closed circuit respiratory system in which carbon dioxide was absorbed by soda-lime. After the chest was opened the same method was used, artificial respiration being carried out under negative pressure ventilation applied to the whole animal.

RESULTS

Experimental conditions of the preparations. When the surgical procedures were finished and perfusion of the brain begun, blood samples were taken from the left and right auricles and analysed for their oxygen and carbon dioxide contents. According to Stewart (1924) the normal range of arteriovenous oxygen differences in dogs is 2·67–5·64 ml./100 ml., and of carbon dioxide 0·45–8·16 ml./100 ml. We have found in our experiments much higher arteriovenous differences which are summarized in Table 1. Despite the fact that the gaseous composition of the arterial blood is normal, the venous blood returns from the tissues much more reduced than normal. This must indicate that cardiac output is very small and/or a certain degree of peripheral circulatory failure exists in these experiments. The blood pressure is always well maintained and therefore any peripheral circulatory failure must be post-arteriolar.

In two experiments, we have therefore determined the cardiac output by the direct Fick method at two different stages of the operative procedure. The first determination was carried out after tracheotomy had been performed and both carotid sinus regions dissected; the second was made after opening the chest in the mid-sternal line and removal of both adrenal glands. The animals were breathing spontaneously during the first determination, and artificial

TABLE 1. Arterial and venous oxygen and carbon dioxide differences and cardiac output determinations. Except in Expts. 21 and 22, the arterial and mixed venous samples were drawn immediately after perfusino of the brain was begun. Open chest; positive pressure ventilation (P.P.V.). N.P.V. = negative pressure ventilation

Expt. no.	Arterial blood contents (ml./100 ml.) O_2	Arterial blood contents (ml./100 ml.) CO_2	Mixed venous blood contents (ml./100 ml.) O_2	Mixed venous blood contents (ml./100 ml.) CO_2	A.-V. O_2 difference	A.-V. CO_2 difference	O_2 consumption (ml./min)	R.Q.	Cardiac output (l./min)	Conditions
5	24·4	25·7	15·45	38·4	8·95	12·7	—	0·70	—	—
17	22·3	34·3	8·1	46·2	14·2	11·9	—	0·84	—	—
18	26·5	27·1	7·87	42·6	18·63	15·5	—	0·83	—	—
19	24·0	29·83	12·85	37·15	11·15	8·32	—	0·74	—	—
20	22·6	31·6	5·8	42·0	16·8	10·4	—	0·62	—	—
21										Wt. of dog, 10·75 kg:
	19·8	49·5	13·9	53·3	5·9	3·8	61	0·65	1·035	11.30 a.m. Spontaneous respiration
	20·6	41·6	8·8	49·5	11·8	7·9	78	0·67	0·667	1.25 p.m. N.P.V. (Chest opened. Adrenalectomized)
	20·9	31·8	5·6	45·8	15·3	14·0	—	0·91	—	2.10 p.m. P.P.V. (10 min after beginning perfusion)
	21·2	29·7	5·4	46·7	15·8	17·0	—	1·07	—	2.58 p.m. P.P.V. (14 min after beginning adrenaline infusion, 2 μg/kg/min)
22										Wt. of dog, 11·25 kg:
	18·1	49·9	12·2	54·4	5·9	4·5	80	0·76	1·337	10.45 a.m. Spontaneous respiration
	18·5	44·6	8·8	51·9	9·7	7·3	70·5	0·75	0·72	1.15 p.m. N.P.V. (Chest opened. Adrenalectomized)
	19·8	39·7	6·5	49·7	13·3	10·0	—	0·75	—	1·35 p.m. P.P.V. (5 min after beginning perfusion)
	20·5	36·2	9·5	44·0	11·0	7·8	—	0·71	—	2.25 p.m. P.P.V. (8 min after beginning adrenaline infusion, 2 μg/kg/min)
	20·2	34·2	9·3	44·7	10·9	10·5	—	0·96	—	2.55 p.m. P.P.V. (Adrenaline infusion continued)
24	24·7	31·0	11·4	41·5	13·3	10·5	—	0·79	—	—

respiration under negative pressure as applied to the whole animal was carried out during the second. The latter conditions facilitate measurement of O_2 consumption in open chest preparations and can be considered comparable to experiments carried out under positive pressure ventiliation as the physiological effects of these two forms of artificial respiration can be considered the same (Maloney & Whittenberger, 1950; Whittenberger & Sarnoff, 1950). The results show that the cardiac output fell by 35·5 and 46 % respectively in the two experiments accompanied by an increase in the arteriovenous oxygen differences.

Further experiments have shown that quantitatively similar changes in arteriovenous oxygen difference and cardiac output can occur when positive pressure respiration is applied to animals breathing spontaneously. It would appear, therefore, that the major cause of these changes is the application of pressure breathing rather than the trauma associated with opening the chest in the mid-sternal line. However, further progressive increases in the arteriovenous oxygen and carbon dioxide differences are probably due to a combination of several factors—positive pressure breathing (Beecher, Bennett & Bassett, 1943; Carr & Essex, 1946; Maloney, Affeldt, Sarnoff & Whittenberger, 1951), trauma due to the extensive surgical procedures and a certain loss of blood through oozing under heparin (Henderson, 1910; Aub & Cunningham, 1920; Root, Walcott & Gregersen, 1947).

In five out of six experiments the isolated perfused lung preparations remained in good condition for periods up to 4 hr with no evidence of pulmonary oedema. In the sixth (Expt. 17), one lobe of the right lung did not ventilate well throughout the experiment, and towards the end, the lungs were collapsing poorly although there was no evidence of oedema fluid in the trachea or larger bronchi. In two out of a total of eighteen dogs whose brains were perfused, oedema was evident in the trachea within 1 hr of beginning the perfusion.

Effects of perfusing the brain with mixed venous blood

When the brain is perfused through the common carotid arteries, with the vertebral arteries occluded, changing the perfusion from left auricular blood to mixed venous blood from the right auricle causes bronchoconstriction (Fig. 2). This effect was observed in twenty-nine tests in seven experiments. In the majority of tests, the switch-over was maintained for periods of 1–2 min. It should be noted that in all our experiments there is a certain time lag between the switch-over and the onset of the response. This is due to the dead space in the pump and connexions (approx. 40 ml.) and therefore represents the time taken for the new blood to traverse it. In *a* (Fig. 2), both carotid sinus nerves were intact, so that the participation of carotid body reflexes in this response cannot be excluded although if due entirely to them, bronchodilatation would be expected (Daly & Schweitzer, 1951). Section of the carotid sinus nerve on

both sides may apparently abolish the bronchoconstrictor effect of mixed venous blood (*b*). This was found to be due not to the abolition of a reflex from the carotid sinus regions, but to an haemodynamic effect: if the perfusion pressure is set at the same level as the systemic blood pressure, then section of the carotid sinus nerves causes a reflex rise in the blood pressure to a level above that of the carotid perfusion pressure. This prevents the perfused blood reaching that part of the brain responsible for the responses in an adequate

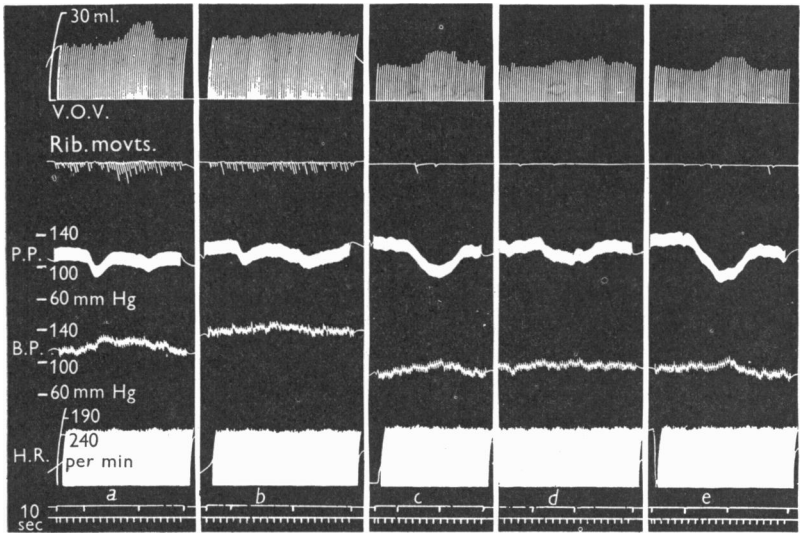

Fig. 2. Dog, ♂, 7·5 kg. Chloralose. Respiratory pump stroke, 130 ml. Perfusion of the brain through the common carotid arteries with blood from the left auricle. Eserine 0·15 mg. Vertebral arteries occluded. *a–e* show bronchomotor effects caused by perfusing the brain with mixed venous blood from the right auricle. Between *a* and *b*, both carotid sinus nerves were cut. The systemic B.P. was then lowered between *b* and *c*, by removal of 30 ml. blood from the femoral artery. The vertebral arteries were released between *c* and *d*, and re-occluded between *d* and *e*. The records illustrate the necessity of maintaining the carotid perfusion pressure higher than the blood pressure and of excluding vertebral blood in order that the perfused blood may reach the brain in adequate concentration. In this and in subsequent figures: V.O.V. = ventilation overflow volume; L.A.P. = left auricular pressure; P.P. = perfusion pressure (carotid or vertebral); B.P. = blood pressure; H.R. = heart rate.

concentration. If the blood pressure is then lowered slightly, by bleeding to a level just below that of the perfusion pressure, bronchoconstriction again results from changing the perfusion from arterial to mixed venous blood (*c*). This illustrates the necessity of maintaining the perfusion pressure at a level higher than the blood pressure in this type of preparation. As the vertebral arteries were occluded, it is probable that the spinal blood was responsible for diluting the perfusion blood, since, as stated previously, its contribution to the blood supply of the brain of the dog is considerable. A similar effect can be

produced by the vertebral blood flow. Changing the perfusion from arterial to mixed venous blood after releasing the vertebral arteries causes a considerable reduction in the bronchomotor response (*d*) which reappears again on occluding the vertebral arteries (*e*).

Eleven experiments have been carried out in which perfusion of the brain was made through the vertebral arteries cannulated near their origin from the subclavian arteries. Responses of the bronchial musculature to changing the perfusion from arterial to mixed venous blood are the same as those obtained when perfusion was made through the common carotid arteries (Fig. 3). Bronchoconstrictor effects were observed in twenty-seven tests, a reduction in tidal air up to 12% being obtained. In a few experiments, there was a concomitant reduction in heart rate, although this was not always very marked (Fig. 5, 1.32 p.m.). This is probably due to stimulation of the vagus centre. The bronchoconstriction is well maintained if perfusion of the brain with mixed venous blood is prolonged. Fig. 4 (*c*) shows the effect of continuing the perfusion for 5 min; changing the perfusion back to arterial blood from the left auricle then releases the bronchoconstriction.

It has been shown that the bronchomotor responses to perfusing the brain with mixed venous blood are little modified by section of the sympathetic nerves innervating the lungs. It would seem, therefore, that alterations in sympathetic tone produced by a central mechanism play a minor part in bringing about these responses. They are, however, abolished by section of the cervical vagosympathetic nerves (three experiments) and by the intravenous injection of atropine (seven experiments) (Figs. 3 and 4). In three out of four experiments, small doses of eserine (20 μg/kg eserine sulphate, B.D.H.) potentiated the bronchoconstrictor responses, while in one further experiment the response was only present after previous injection of eserine.

In many respects, these perfusions through the vertebrals gave more consistent results than those carried out through the carotid arteries as slightly larger blood flows were obtained, and from the anatomical arrangements of the blood vessels, it would seem probable that less mixing of the perfusion blood with blood from the spinal arteries takes place. Some support for this has been obtained by injecting indian ink into the inflow tubing to the vertebral arteries and then, 5–8 sec later, simultaneously tying a stout ligature round the atrioventricular groove of the heart and switching off the perfusion pump. Dissection of these specimens showed indian ink in all parts of the brain and spinal cord to a level of C6, the occipital and cervical muscles and parts of the mouth. These perfused regions are essentially the same as those found by Schmidt (1928) who also perfused the cerebral vessels through the vertebrals after tying off the common carotid arteries.

Effect of altering the perfusion pressure. In most experiments, a fall in perfusion pressure occurs on perfusing the brain with mixed venous blood.

Since the blood volume inflow is constant, this must be due to a reduction in peripheral resistance. It has been shown previously that the vagus centre is not in itself sensitive to changes in pressure when the heart rate is used as

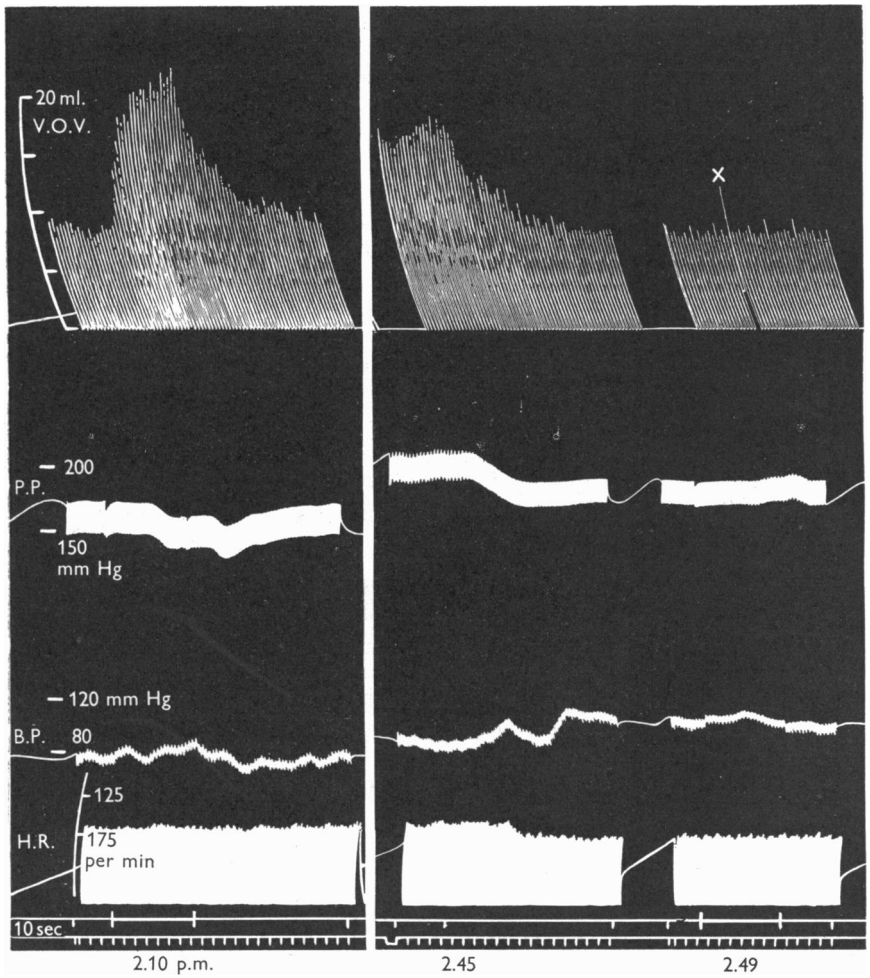

Fig. 3. Dog, ♂, 11·25 kg. Nembutal. Adrenalectomized. Respiration pump stroke, 175 ml. Carotid circulation tied off. Both carotid sinus nerves sectioned. Adrenaline infusion 20 μg/min. Perfusion of brain through the vertebral arteries with blood from the left auricle (pO_2 100+, pCO_2 45 mm Hg, pH 7·37) begun at 1.35 p.m. At 2.10 and 2.49 p.m., perfusion of the brain with blood from the right auricle (pO_2 22, pCO_2 53 mm Hg, pH 7·28). At 2.45 p.m., atropine, 5 mg, injected intravenously.

the indicator (Heymans, Bouckaert & Moraes, 1932). Varying the pressure from 60 to 210 mm Hg by altering the output of the pump does not cause any change in heart rate or in the ventilation overflow volume. In our preparations

the vertebral or carotid perfusion pressure cannot be lowered below 60 mm Hg (approx.), even with the pump switched off, owing to the back-flow from the spinal arteries (Chungcharoen *et al.* 1952). Similar tests were carried out when the brain was being perfused with mixed venous blood instead of arterial blood. Lowering the perfusion pressure from its pre-set level of about 160 to 60 mm Hg,

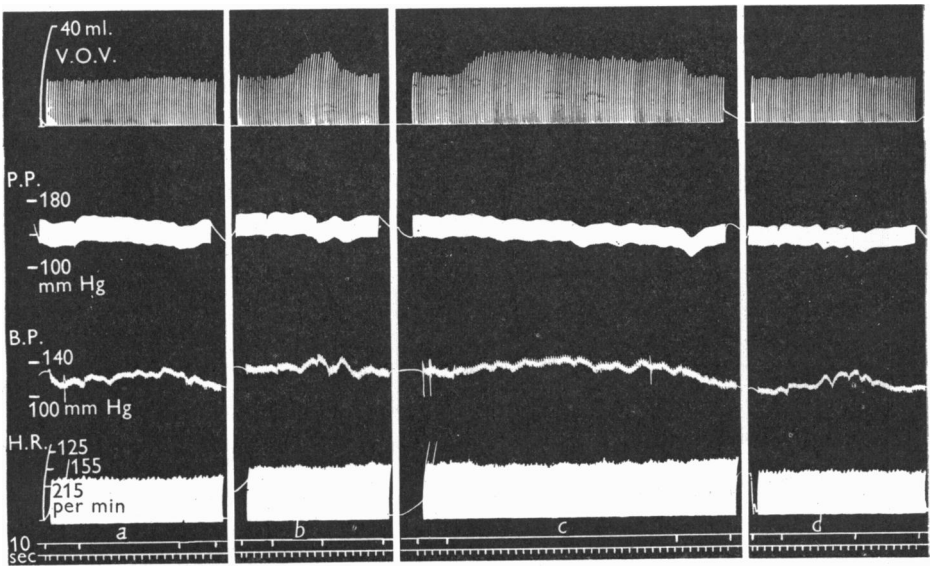

Fig. 4. Dog, ♂, 9 kg. Chloralose. Respiration pump stroke, 165 ml. Both carotid sinus nerves cut. Carotid circulation tied off. Perfusion of the brain through the vertebral arteries with blood from the left auricle. *a–d* show the effects of changing the perfusion to mixed venous blood from the right auricle. Between *a* and *b*, eserine, 0·15 mg intravenously. Between *c* and *d*, atropine, 5 mg intravenously.

causes release of the bronchoconstriction. We interpret this as being due to a progressive dilution of the perfused blood with spinal arterial blood as the pressure is reduced and not to a pressure effect on the vagus centre. This phenomenon has already been referred to above. The fall in perfusion pressure will therefore have no direct influence on the vagus centre other than by altering the amount of mixing of perfusion blood with blood from the spinal arteries.

In some experiments, changes in movements of the ribs were recorded in response to changing the gaseous composition of the blood perfusing the brain. An increase in movements due to stimulation of the respiratory centre usually occurs when arterial blood is changed for mixed venous blood. The concomitant changes in ventilation overflow volume might therefore be considered to be a mechanical effect on the lungs due to movements of the thorax. However, this appears unlikely as the changes in ventilation overflow volume did not bear any relationship to the changes in rib movements (Fig. 2).

Further, when slightly hyperventilating the animal so as to abolish rib movements, bronchoconstrictor responses to changing the perfusion from arterial to mixed venous blood still occur.

In a few experiments, the pressure in the left auricle has been simultaneously recorded by means of a volume recorder connected to the open end of a vertical saline manometer (McDowall, 1922). The pressure changes observed have not been greater than 2 cm saline on switching the perfusion from arterial to mixed venous blood. Injection of blood into the left auricle at a rate sufficient to cause a similar rise in left auricular pressure did not produce an increase in ventilation overflow volume. Furthermore, changes in ventilation overflow volume were not always in the same direction as the changes in left auricular pressure (Fig. 6). Larger changes than this may cause a diminution in tidal air (Drinker, Peabody & Blumgart, 1922; Bulbring & Whitteridge, 1945), although effects on the ventilation overflow volume, using the method of Konzett & Rössler (1940), are not evident in isolated perfused dog lungs until the left auricular pressure is raised by 6–9 cm saline (Daly, 1951, unpublished observations).

Since bronchomotor responses to mixed venous blood perfusion were abolished by atropinization and by vagotomy, we are confident that lung blood volume changes are not wholly responsible for the records obtained, particularly as such changes must still occur after these experimental procedures due to redistribution of blood from the systemic circulation.

From the evidence so far presented, it might appear conclusive that the bronchomotor responses are due to a direct central action of the perfused blood. Two other possible explanations, however, must be taken into consideration. Alteration in cerebral blood gas composition such as that produced by cerebral anaemia causes the secretion of adrenaline. It has been shown by Viale (1928, 1930) and by Stella (1932) that adrenaline may modify the vasomotor reflexes from the baroreceptors by an action on the medullary centres. Thus, in our own experiments, the bronchomotor responses could be due to a modification of the normal aortic baroreceptor discharge by a similar mechanism. That this is not so has been shown in preparations in which both the suprarenal glands were removed; in three experiments, quantitatively similar responses to perfusing the brain with mixed venous blood were observed (Fig. 3).

A possible reflex effect by an action on chemoreceptors in the lungs, postulated by Pi-Suner (1947), due to change in gas tensions in the blood returning to the right heart from the brain has also been excluded. Tests carried out by perfusing the right side of the heart through the right auricle with either anoxic or hypocapnic blood at the same blood volume inflow as that which was used to perfuse the brain have not produced any bronchomotor effects.

It is clear, therefore, that the bronchomotor responses observed on per-

fusing the brain with mixed venous blood are central in origin and mediated through the vagus nerves. Experiments were then designed with a view to analysing the effect of mixed venous blood by altering the oxygen and carbon dioxide tensions of the blood perfusing the brain separately.

Effects of altering the oxygen and carbon dioxide tensions of the blood perfusing the brain

Five experiments were carried out in which the effects were observed of changing the composition of the blood perfusing the brain from arterial blood to blood equilibrated in the isolated perfused lungs of a second dog with various gas mixtures (Fig. 1). The usual procedure was first to observe the response to switching over to right auricular blood, and then to perfusing the brain with blood equilibrated with a mixture of O_2 and 5 or 6 % CO_2. Blood equilibrated in this way had oxygen and carbon dioxide tensions practically the same as those of arterial blood of the dog with only small differences in the pCO_2 in two of the experiments. Thereafter, tests were made in which the oxygen and carbon dioxide tensions of the blood were varied independently. The brain was perfused with arterial blood from the left auricle, and the individual tests were made by switching over to the inner reservoir *f* (Fig. 1) for periods of 1–2 min after which the perfusion was switched back again to left auricular blood (Table 2). The gas tensions and pH of the blood of the individual tests should therefore be compared with those of the left auricular blood.

Perfusion with equilibrated arterial blood. In each experiment tests were made with blood equilibrated with a mixture of O_2 and 5 or 6 % CO_2. In Expt. 17 (Table 2) no bronchomotor effect resulted, despite the fact that the pCO_2 of the equilibrated arterial blood was lower than that of the left auricular blood. The high arterial pCO_2 was in all probability due to the animal being underventilated. Of the other four experiments, a small increase in the ventilation overflow volume was noted in two of them (Expts. 18 and 19) where a large difference existed between the pCO_2 of the equilibrated blood and that of the left auricular blood. As it has been shown that an increase in carbon dioxide tension causes bronchoconstriction, the slight effects observed in these two experiments can be explained on this basis. It is concluded, therefore, that when blood is equilibrated with a gas mixture to give similar oxygen and carbon dioxide tensions as exist in the arterial blood of the test dog, no bronchomotor effect results. This rules out the possibility that the responses obtained by perfusing the brain with 'shed' blood are due to circulating vasotonins or bronchotonins (I. de B. Daly, 1938).

Perfusion with anoxic and hypercapnic blood. When blood is equilibrated with a mixture of 5 % O_2 and 10 % CO_2 and perfused through the brain,

bronchoconstriction occurs (four tests in four experiments). These results are the same therefore as those obtained with mixed venous blood.

Perfusion with anoxic blood. Perfusion of the brain with blood equilibrated with gas mixtures containing 5 % O_2 and 5 or 6 % CO_2 causes bronchoconstriction. This response was obtained in six tests in four experiments (Fig. 5, 1.46 p.m. and Table 2). In one further test (Expt. 18) no response was observed. It will be noted that the pO_2 was 64 mm Hg, whereas in the other tests, the

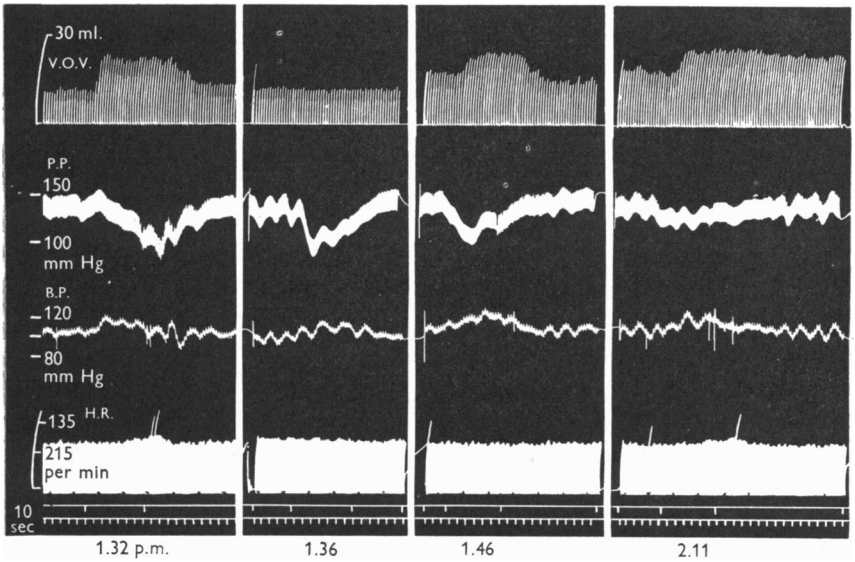

Fig. 5. Dog, ♂, 11·0 kg. Chloralose. Respiration pump stroke, 160 ml. Both carotid sinus nerves sectioned. Carotid circulation tied off. Blood pressure maintained constant with compensator. Perfusion of the brain through the vertebral arteries with blood from the left auricle (pO_2 100 +, pCO_2 53 mm Hg, pH 7·31) begun at 1.20 p.m. Isolated perfused lungs of a second dog (10 kg), bled under Nembutal, was used for the equilibration of blood. At 1.32 p.m., change-over to mixed venous blood from the right auricle (pO_2 30, pCO_2 70 mm Hg, pH 7·10). At 1.36 p.m., test with blood equilibrated with 95 % O_2 and 5 % CO_2 (pO_2 75, pCO_2 34 mm Hg, pH 7·17). At 1.46 a.m., change-over to anoxic blood equilibrated with 5 % O_2, 5 % CO_2 and 90 % N_2 (pO_2 43, pCO_2 30 mm Hg, pH 7·23). At 2.11 p.m., change-over to hypercapnic blood equilibrated with 90 % O_2 and 10 % CO_2 (pO_2 95, pCO_2 90 mm Hg, pH 6·88).

tension was lower, 33–43 mm Hg (Table 2). These results confirm those obtained previously in experiments in which anoxic blood, injected into the common carotid artery, caused bronchoconstriction (Daly & Schweitzer, 1952).

Perfusion with hypercapnic blood. Blood equilibrated with a mixture of 90 % O_2 and 10 % CO_2 in the isolated perfused lungs caused bronchoconstriction when perfused through the brain (Fig. 5). This result was obtained in six tests in four experiments. In one experiment (Expt. 20) no effect occurred

TABLE 2. The effect of perfusing the brain with mixed venous blood from the right auricle and with blood equilibrated with various gas mixtures in the isolated perfused lungs of a second dog. The brain was perfused with blood from the left auricle, the oxygen and carbon dioxide tensions and pH of which are given below. Each test was carried out for a period of 1–2 min after which the perfusion is switched back to blood from the left auricle.

Expt. no. ...		17				18				19				20				23			
	Equilibrating gas mixture	Blood gas tensions, mm Hg				Blood gas tensions, mm Hg				Blood gas tensions, mm Hg				Blood gas tensions, mm Hg				Blood gas tensions, mm Hg			
		pO_2	pCO_2	pH	B.R.	pO_2	pCO_2	pH	B.R.	pO_2	pCO_2	pH	B.R.	pO_2	pCO_2	pH	B.R.	pO_2	pCO_2	pH	B.R.
Left auricle blood	—	100+	53	7·31	—	100+	26	7·37	—	100+	22	7·48	—	100+	31	7·34	—	—	—	—	—
Right auricle blood	—	30	70	7·10	C	24	58	7·20	C	30	35	7·35	C	18	45	7·29	C	28	52	7·27	C
Equilibrated arterial blood	O_2 and 5 or 6 % CO_2	75	34	7·17	None	100+	39	7·29	Sl.C	100+	39	7·22	C	100+	34	7·34	None	100+	36	7·34	None
Anoxic and hypercapnic	5 % O_2 and 10 % CO_2	—	—	—	—	38	95	7·0	C	48	110	6·73	C	42	53	7·20	C	43	52	7·24	C
										approx.											
Anoxic	5 % O_2 and 5 or 6 % CO_2	43	30	7·23	C	64	30	7·41	None	36	34	7·34	C	36	39	7·31	C	33	34	7·38	C
Hypercapnic	90 % O_2 and 10 % CO_2	95	90	6·88	C	100+	66	7·15	C	100+	51	7·22	C	100+	40	7·30	None	100+	70	7·14	C
Hypocapnic	Atmospheric air 100 % O_2	46	18	7·30	D	100+	12	7·64	D	100+	5	7·64	D	100+	5	7·68	D	100+	13	7·53	D
Anoxic and hypocapnic	5 % O_2 and 95 % N_2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	43	19	7·46	Sl.C
Atropine	—	—	—	—	D	—	—	—	—	—	—	—	D	—	—	—	D	—	—	—	D
Section of cervical vago-sympathetic nerves	—	—	—	—	—	—	—	—	D	—	—	—	—	—	—	—	—	—	—	—	—

The figures given for Expt. 17 suggest that some parts of the isolated perfused lung were not being ventilated. C = bronchoconstriction; D = bronchodilatation; Sl. = slight. B.R. = bronchomotor response.

as the pCO_2 of the perfused blood was only 40 mm Hg, probably through an error in preparing the gas mixture. Traube (1865) first showed that excess CO_2 caused stimulation of the vagus centre, and in two of our own experiments, there was a concomitant bradycardia (Fig. 5, 2.11 p.m.). Thus blood containing a raised carbon dioxide tension, and with it a diminished pH, stimulates the vagus centre causing bronchoconstriction and bradycardia.

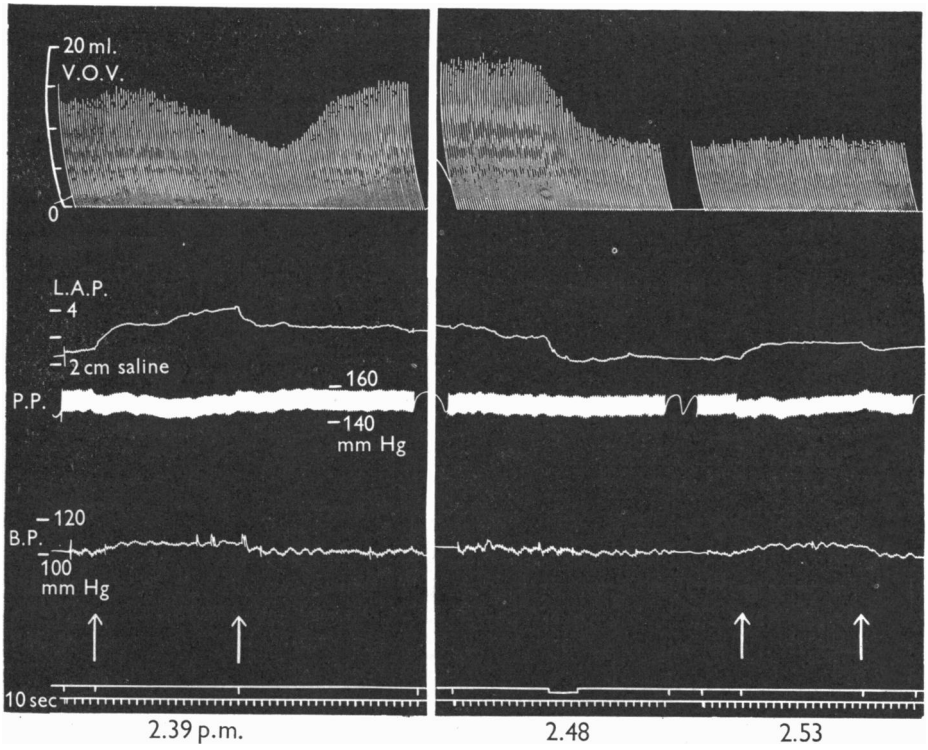

Fig. 6. Dog, ♀, 11·3 kg. Chloralose. Respiration pump stroke, 180 ml. Both carotid sinus nerves sectioned. Carotid circulation tied off. Perfusion of the brain through the vertebral arteries with blood from the left auricle begun at 1.12 p.m. Isolated perfused lungs of a second dog (13 kg), bled under morphia, was used for the equilibration of blood. At 2.39 and 2.53 p.m., perfusion with left auricular blood (pO_2 100+, pCO_2 22 mm Hg, pH 7·48) changed for blood equilibrated with 100 % O_2 (pO_2 100+, pCO_2 5 mm Hg, pH 7·64). At 2.48 p.m., atropine, 5 mg, injected intravenously.

The bronchoconstrictor effects produced by perfusing the brain with either mixed venous, anoxic and hypercapnic, anoxic or hypercapnic blood are abolished by atropine (four experiments) and by section of the cervical vagosympathetic nerves (one experiment). Atropine itself, in these four experiments, caused bronchodilatation, as did section of the cervical vagosympathetic nerves in the remaining one.

Perfusion with hypocapnic blood. It has been shown that the vagus exerts a tonic influence on the bronchial musculature (Roy & Brown, 1885; Chauveau, 1889; Daly & Mount, 1951) which is maintained, in part, by baroreceptor activity (Daly & Schweitzer, 1952). Since it has been shown in the present investigation that an increase in the carbon dioxide tension of the blood perfusing the brain causes bronchoconstriction, it was wondered if the normally existing tone would be influenced by a diminution in carbon dioxide tension.

Blood was equilibrated with atmospheric air in one experiment and with 100 % O_2 in four others and perfused through the brain. In seven tests in the five experiments, changing the perfusion from left auricular blood to hypocapnic blood resulted in bronchodilatation (Fig. 6, 2.39 p.m. and Table 2). If now the tone is abolished by the injection of atropine or by section of the cervical vagosympathetic nerves, no further bronchomotor effect is observed (2.53 p.m.). The initial response in two other tests were negative. Other experiments have shown that bronchodilator responses occur in preparations in which the sympathetic nerves innervating the lungs have been eliminated (excision of the stellate ganglia and sympathetic chains as far as T5) and both adrenal glands removed. They must therefore be due mainly to a diminution of vagus tone.

It will be noted that in Expt. 17, a bronchodilator response occurred despite the oxygen tension of the blood being as low as 46 mm Hg. The low pO_2 was probably due to the poor condition of the isolated perfused lung in this experiment. Nevertheless, it shows that the bronchodilator effect of the low pCO_2 predominates over the bronchoconstrictor effect of the low pO_2. In one other experiment, a test was carried out in which the blood was equilibrated with a mixture of 5 % O_2 in nitrogen. When this blood was perfused through the brain, a slight bronchoconstriction resulted, so that the pO_2 which was slightly lower than in the above experiment just antagonized the bronchodilator effect of the low pCO_2 (Table 2). That tone was in fact present in this experiment was shown by a subsequent injection of atropine which caused bronchodilatation. The experiments illustrate, therefore, that both low pO_2 and low pCO_2 produce central antagonistic effects. The evidence for the effects of oxygen lack and carbon dioxide excess summating due to a central action is suggestive but less convincing (Fig. 5).

DISCUSSION

We have shown that changes in bronchial tone occur when the oxygen and carbon dioxide tensions of the blood perfusing the brain are altered. If the blood perfusing the brain is changed from arterial to mixed venous composition, bronchoconstriction results; an analysis shows that this effect is due to both the diminished oxygen tension and raised carbon dioxide tension of the blood. In some experiments, there was a concomitant bradycardia. Perfusion of the

brain with hypocapnic blood causes bronchodilatation. Both the bronchoconstrictor and bronchodilator effects have been shown to be mediated mainly through the vagus nerves and must therefore be brought about through alterations in vagus tone.

We are not in a position to state which part of the brain is responsible for the observed effects as the regions perfused included the whole brain and upper part of the spinal cord.

Cerebral anaemia causes bradycardia (Neujean, 1904; Heymans & Ladon, 1925) and bronchoconstriction (Houssay & Cruciani, 1929; Daly & Schweitzer, 1952), both these effects being abolished by section of the vagus nerves. Excess carbon dioxide stimulates the vagus centre, again causing bradycardia (Traube, 1865; Hill & Flack, 1908) and bronchoconstriction (Einthoven, 1892; Dixon & Brodie, 1903). The results of the present investigation confirm the main conclusions of the last-mentioned workers and show further that anoxic blood causes a similar bronchomotor effect by a central action. Moreover, by maintaining the oxygen tension normal and reducing the carbon dioxide tension of the blood perfusing the brain, bronchodilatation results; hypocapnia also causes tachycardia due to a diminution of vagus tone (Bainbridge, 1920; Heymans, 1929). Thus the results we have obtained from a study of central effects of anoxia, hypercapnia and hypocapnia upon the bronchioles agree with those of other workers studying the same effects on the heart rate and suggest that, in both cases, the effects are probably due to an action on the vagus centre. We hesitate, in the absence of any evidence, to postulate separate cardio-inhibitory and bronchomotor 'centres'.

When studying the central effects of changes in blood gas tensions upon the vagus and vasomotor tone, Heymans, Bouckaert & Samaan (1934) distinguished two effects: a direct one upon the centres in the absence of impulses from the vasosensory areas of the carotid sinus and arch of the aorta, and a change of the sensitivity of a carotid sinus and aortic reflex. It could be argued that the bronchomotor responses which we have observed are not caused by a direct action on the vagus centre *per se*, but to a central modification of the normal baroreceptor discharge in fibres in the aortic nerves which were left intact. This possibility cannot be denied, but whichever view is taken, it does not detract from the effects being central in origin. No attempt was made to repeat any of the experiments after section of both the carotid sinus and aortic nerves, for not only are the latter nerves difficult to find in the dog (Green, Degroat & McDonald, 1935; Daly & Schweitzer, 1952), but even if they were sectioned there would remain afferent fibres from other regions making connexion with the vagus centre. It is probably impossible, therefore, to de-afferentate the vagus centre completely and yet leave the efferent vagus fibres to the heart and lungs intact.

In their investigation of the central effects upon heart rate, Heymans *et al.*

(1934) concluded that only pathological changes in inspired gas concentrations would stimulate the vagus centre. An inspection of the figures presented in our Table 2 of the changes in cerebral inflow blood gas tensions necessary to bring about bronchomotor responses would suggest that they are outside the normal physiological range. However, there are two points worth mentioning which favour the view that these values are higher than those which might be required in preparations under more normal conditions to produce bronchomotor responses. The medullary centres are probably partially depressed by the anaesthetic and the general circulatory state of our preparations, the cause of which has already been referred to earlier in the paper. Moreover, the perfused blood may be mixed to a small extent with blood from the spinal arteries which would tend to diminish its effectiveness by dilution before reaching the capillaries. No attempts have been made to estimate the changes in cerebral venous blood gas tensions in these experiments.

When considering the results of this investigation teleologically, it would appear disadvantageous if, under an adverse condition such as asphyxia, bronchoconstriction resulted. For a number of reasons, we do not think that this argument necessarily holds. The responses which have been observed are those elicited from only one part of the organism, namely, the brain. Changes in blood gas tension affecting the whole organism, such as are produced by altering the inspired air gas tensions will, in all probability, bring into play other mechanisms affecting the bronchioles. The bronchial musculature is influenced directly by changes in inspired air gas tensions, both oxygen lack and excess carbon dioxide causing bronchodilatation (Löhr, 1924; Nisell, 1950; Duke, 1951). The same result would be expected from similar changes in blood gas tensions acting reflexly through stimulation of the carotid and aortic chemoreceptors (Daly & Schweitzer, 1951). These effects, therefore, are opposite to those found in the present investigation in response to the same directional changes in cerebral blood gas tensions. Concomitant blood pressure changes will also influence the bronchioles reflexly through the baroreceptors (Daly & Schweitzer, 1951, 1952) while reflex secretion of adrenaline may be effective by direct action. In addition, the sensitivity of some of the reflexes mentioned may be modified both centrally and/or peripherally by changes in blood gas tensions (Vercauteren, 1932; Heymans *et al.* 1934). The bronchomotor responses in the intact animal to changes in inspired gas tensions will, therefore, depend on a number of factors, not least on the magnitude of the stimulus applied.

Whatever may be the effects of small changes in blood gas tensions, the results of Roy & Brown, Einthoven, and of Dixon & Brodie do suggest that profound changes cause bronchoconstriction. Thus, in asphyxia a state might be reached whereby the blood becomes so reduced that bronchoconstriction occurs, resulting in a further deleterious effect on the organism. Such an effect

might be expected to occur in the train of events produced by acute poisoning due to an anticholinesterase in which asphyxia may be a prominent feature through failure of the respiratory mechanism and a varying degree of bronchoconstriction. Thus a vicious circle would be set up. The experiment illustrated in fig. 3 of a previous paper may be reinterpreted in this light (Daly & Schweitzer, 1951). The bronchoconstriction due to stimulation of the carotid sinus nerve after an intravenous injection of eserine was so severe that the preparation became asphyxiated as indicated by the decreased oxygen uptake, rise of blood pressure and the slowing of the heart rate. The gradual onset of bradycardia and increasing bronchoconstriction after the electrical stimulus would suggest that these effects were primarily of central origin, although a reflex contribution to the effects by the blood pressure rise cannot be completely ruled out.

In a previous investigation, we made a study of the reflex control of the tone of the bronchial musculature (Daly & Schweitzer, 1952). The technique used was to lower the blood pressure in the carotid sinus and aortic regions by subjecting the animals to acute haemorrhage followed by transfusion of the blood. Bronchodilatation resulted on lowering the pressure. Under these conditions changes in the oxygen and carbon dioxide tensions of the tissues might be expected to occur (Hertzman & Gesell, 1927). The possibility of this response being central in origin through anaemia was, therefore, ruled out by the use of several methods, one of which was to inject blood containing different gas tensions into the common carotid artery. Both oxygen lack and the excess carbon dioxide caused bronchoconstriction through a central action. These findings are fully confirmed by the results of the present investigation.

Several workers, already cited, have shown that the bronchial musculature is tonically innervated by the vagus nerves. We have shown previously that this tone is, in part, maintained reflexly by the activity of the baroreceptors of the vasosensory areas (Daly & Schweitzer, 1952), and the present experiments throw further light on the mechanism of its maintenance. Since perfusion of the brain with hypercapnic blood causes bronchoconstriction, and hypocapnic blood diminishes vagal tone resulting in bronchodilatation, it is concluded that the normal carbon dioxide tension of the blood must contribute to the maintenance of bronchomotor tone by a central action.

SUMMARY

1. The central control of bronchomotor tone has been investigated in the dog. A technique of perfusing the brain through the common carotid or vertebral arteries with either arterial blood from the left auricle or mixed venous blood from the right auricle is described.

2. Changing the perfusion from arterial to mixed venous blood causes bronchoconstriction. This response is potentiated by eserine and abolished by

atropine, and by section of the cervical vagosympathetic nerves. As the carotid sinus regions are denervated, the responses are central in origin.

3. An analysis of the bronchoconstrictor effect of mixed venous blood has been made by changing the perfusion from arterial blood to blood equilibrated with various gas mixtures in the isolated perfused lungs of a second dog. Passing either anoxic and/or hypercapnic blood through the brain causes bronchoconstriction. Hypocapnic blood causes bronchodilatation, an effect which is due to a diminution of vagus tone.

4. It is concluded that the normal carbon dioxide tension of the blood contributes to the maintenance of vagal bronchomotor tone.

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REFERENCES

AUB, J. C. & CUNNINGHAM, T. D. (1920). Studies in experimental traumatic shock. II. The oxygen content of the blood. *Amer. J. Physiol.* **54**, 408–415.

BAINBRIDGE, F. A. (1920). The relation between respiration and the pulse-rate. *J. Physiol.* **54**, 192–202.

BEECHER, H. K., BENNETT, H. S. & BASSETT, D. L. (1943). Circulatory effects of increased pressure in the airway. *Anesthesiology*, **4**, 612–618.

BRAEUCKER, W. (1926). Die experimentelle Erzeugung des Bronchialasthmas und seine operative Beseitigung. *Arch. klin. Chir.* **139**, 1–63.

BÜLBRING, E. & WHITTERIDGE, D. (1945). Activity of vagal stretch endings during congestion in perfused lungs. *J. Physiol.* **103**, 477–487.

CARR, D. T. & ESSEX, M. E. (1946). Certain effects of positive pressure respiration on the circulatory and respiratory systems. *Amer. Heart J.* **31**, 53–73.

CHAUVEAU (1889). Des troubles respiratoires consecutifs à la section des pneumogastriques. *Sem. médicale*, p. 117.

CHUNGCHAROEN, D., DALY, M. DE BURGH, NEIL, E. & SCHWEITZER, A. (1952). The effect of carotid occlusion upon the intrasinusal pressure with special reference to vascular communications between the carotid and vertebral circulations in the dog, cat and rabbit. *J. Physiol.* **117**, 56–76.

DALE, H. H. & SCHUSTER, E. H. J. (1928). A double perfusion-pump. *J. Physiol.* **64**, 356–364.

DALY, I. DE BURGH (1938). Observations on the blood perfused lungs of the dog, guinea-pig and *Macacus rhesus*, with special reference to 'spontaneous' lung movements. *Quart. J. exp. Physiol.* **28**, 357–403.

DALY, I. DE BURGH & DUKE, H. (1948). Note on a method for the demonstration of pulmonary vasomotor fibres. *Quart. J. exp. Physiol.* **34**, 151–158.

DALY, I. DE BURGH & HEBB, C. O. (1942). Bronchomotor and pulmonary arterial pressure responses to nerve stimulation. *Quart. J. exp. Physiol.* **31**, 211–226.

DALY, M. DE BURGH, LAMBERTSEN, C. J. & SCHWEITZER, A. (1952). Bronchomotor responses to altering the gaseous composition of the blood perfusing the brain. *J. Physiol.* **117**, 60 *P*.

DALY, M. DE BURGH & MOUNT, L. E. (1951). The origin, course and nature of bronchomotor fibres in the cervical sympathetic nerve of the cat. *J. Physiol.* **113**, 43–62.

DALY, M. DE BURGH & SCHWEITZER, A. (1950). A method of recording heart-rate on the kymograph. *J. Physiol.* **111**, 50–52 *P*.

DALY, M. DE BURGH & SCHWEITZER, A. (1951). Reflex bronchomotor responses to stimulation of receptors in the regions of the carotid sinus and the arch of the aorta in the dog and cat. *J. Physiol.* **113**, 442–462.

DALY, M. DE BURGH & SCHWEITZER, A. (1952). The contribution of the vasosensory areas to the reflex control of bronchomotor tone. *J. Physiol.* **116**, 35–58.

DIXON, W. E. & BRODIE, T. G. (1903). Contribution to the physiology of the lungs. Part I. The bronchial muscles, their innervation, and the action of drugs upon them. *J. Physiol.* **29**, 97–173.

DIXON, W. E. & RANSOM, F. (1912). Bronchodilator nerves. *J. Physiol.* **45**, 413–428.

DRABKIN, D. L. & AUSTIN, J. H. (1935). Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *J. biol. Chem.* **112**, 51–65.

DRINKER, C. J., PEABODY, F. W. & BLUMGART, H. (1922). The effect of pulmonary congestion on the ventilation of the lungs. *J. exp. Med.* **35**, 77–95.

DUKE, H. N. (1951). Pulmonary vasomotor responses of isolated perfused cat lungs to anoxia and hypercapnia. *Quart. J. exp. Physiol.* **36**, 75–88.

EINTHOVEN, W. (1892). Ueber die Wirkung der Bronchialmuskeln, nach einer neuen Methode untersucht, und über Asthma nervosum. *Pflüg. Arch. ges. Physiol.* **51**, 367–445.

EVANS, C. L. & SAMAAN, A. (1936). Simultaneous ligature of the carotid and vertebral arteries in the dog. *J. Physiol.* **87**, 33–34 *P.*

GADDUM, J. H. & KWIATKOWSKI, H. (1938). The action of ephedrine. *J. Physiol.* **94**, 87–100.

GADDUM, J. H., PEART, W. S. & VOGT, M. (1949). The estimation of adrenaline and allied substances in blood. *J. Physiol.* **108**, 467–481.

GREEN, M. F., DEGROAT, A. F. & MCDONALD, C. H. (1935). Observations on denervation of the carotid sinuses and section of the depressor nerves as a method of producing arterial hypertension. *Amer. J. Physiol.* **110**, 513–520.

HEBB, C. O. & LINZELL, J. L. (1951). Some conditions affecting the blood flow through the perfused mammary gland, with special reference to the action of adrenaline. *Quart. J. exp. Physiol.* **36**, 159–175.

HENDERSON, Y. (1910). Acapnia and shock. VII. Failure of the circulation. *Amer. J. Physiol.* **27**, 152–176.

HERTZMAN, A. B. & GESELL, R. (1927). The regulation of respiration. IX. The relation of tissue-acidity and blood-acidity to volume flow of blood as illustrated by hemorrhage and reinjection. *Amer. J. Physiol.* **81**, 563–578.

HEYMANS, C. (1929). Über die Physiologie und Pharmakologie des Herz-Vagus-Zentrums. *Ergebn. Physiol.* **28**, 244–311.

HEYMANS, C., BOUCKAERT, J. J. & MORAES, A. (1932). Perfusion purement artificielle de la circulation céphalique 'isolée'. Réactions vasomotrices. *C.R. Soc. Biol., Paris*, **109**, 1138–1141.

HEYMANS, C., BOUCKAERT, J. J. & SAMAAN, A. (1934). Influences des variations de la tenseur du sang en oxygène et en CO_2 sur l'excitabilité réflexe et directe des éléments centraux et périphériques des nerfs cardio-régulateurs. *Arch. int. Pharmacodyn.* **48**, 457–487.

HEYMANS, C. & LADON, A. (1925). Recherches physiologiques et pharmacologiques sur la tête isolée et le centre vague du chien. *Arch. int. Pharmacodyn.* **30**, 415–453.

HILL, L. (1896). *The Physiology and Pathology of the Cerebral Circulation.* London: Churchill.

HILL, L. & FLACK, M. (1908). The effect of excess carbon dioxide and want of oxygen upon the respiration and the circulation. *J. Physiol.* **37**, 77–111.

HOUSSAY, B. A. & CRUCIANI, J. (1929). Étude des actions centrales ou périphériques sur les bronches chez le chien, à tête isolée. *C.R. Soc. Biol., Paris*, **101**, 246–248.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch. exp. Path. Pharmak.* **195**, 71–74.

LÖHR, H. (1924). Untersuchungen zur Physiologie und Pharmakologie der Lunge. *Z. ges. exp. Med.* **39**, 67–130.

MALONEY, J. V., AFFELDT, J. E., SARNOFF, S. J. & WHITTENBERGER, J. L. (1951). Electrophrenic respiration. The comparison of effects of positive pressure breathing and electrophrenic respiration on the circulation during haemorrhage shock and barbiturate poisoning. *Surg. Gynec. Obstet.* **92**, 672–684.

MALONEY, J. V. & WHITTENBERGER, J. L. (1950). Circulatory effects of the body respirator. *Amer. J. Med.* **8**, 393.

MCDOWALL, R. J. S. (1922). An easy method of recording venous pressure. *J. Physiol.* **57**, 5 *P.*

NEUJEAN, V. (1904). Contribution à l'étude expérimentale de l'adrenaline. *Arch. int. Pharmacodyn.* **13**, 45–90.

NISELL, O. I. (1950). The action of oxygen and carbon dioxide on the bronchioles and vessels of the isolated perfused lungs. *Acta physiol. scand.* **21**, Suppl. 73.

PI-SUNER, A. (1947). The regulation of the respiratory movements by peripheral chemoreceptors. *Physiol. Rev.* **27**, 1–38.

ROBERTS, F. (1921). The effect of adrenaline upon respiration. *J. Physiol.* **55**, 346–355.

ROOT, W. S. WALCOTT, W. W. & GREGERSEN, M. I. (1947). Effects of muscle trauma and of haemorrhage upon the cardiac output of the dog. *Amer. J. Physiol.* **151**, 34–44.

ROSENTHAL, T. B. (1948). The effect of temperature on the pH of blood and plasma *in vitro*. *J. biol. Chem.* **173**, 25–30.

ROY, C. S. & BROWN, G. (1885). On bronchial contraction. *J. Physiol.* **6**, 21–25 *P*.

SCHMIDT, C. F. (1928). The influence of cerebral blood flow on respiration. I. The respiratory responses to changes in cerebral blood-flow. *Amer. J. Physiol.* **84**, 202–222.

STELLA, G. (1932). The action of adrenaline upon the cardiac vagus centres. *J. Physiol.* **77**, 68–80.

STEWART, H. J. (1924). The oxygen and carbon dioxide contents of the arterial and mixed venous blood in normal intact dogs. *J. biol. Chem.* **62**, 641–647.

TRAUBE, L. (1865). Ueber periodische Thätigeits-Aeusserungen des vasomotorischen und Hemmungs-Nervencentrums. *Zbl. med. Wiss.* **3**, 881–885.

VAN SLYKE, D. D. & NEILL, J. M. (1924). The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. I. *J. biol. Chem.* **61**, 523–573.

VAN SLYKE, D. D. & SENDROY, J., JR. (1928). Studies of gas and electrolyte equilibria in blood. XV. Line charts for graphic calculations by the Henderson-Hasselbalch equation, and for calculating plasma carbon dioxide content from whole blood content. *J. biol. Chem.* **79**, 781–798.

VAN SLYKE, D. D., SENDROY, J., JR. & LUI, S. H. (1932). Manometric analysis of gas mixtures. III. Manometric determination of carbon dioxide tension and pH of blood. *J. biol. Chem.* **95**, 547–567.

VERCAUTEREN, E. (1932). Recherches sur la pharmacologie des réflexes vasomoteurs du sinus carotidien. *Arch. int. Pharmacodyn.* **42**, 339–385.

VIALE, G. (1928). Le tonus vagal, fonction de l'adrenalinémie. *C.R. Soc. Biol., Paris*, **99**, 2008–2009.

VIALE, G. (1930). La funzione vagale nella insufficienza surrenale. *Arch. Fisiol.* **28**, 9–24.

WHITTENBERGER, J. L. & SARNOFF, S. J. (1950). Physiologic principles in the treatment of respiratory failure. *Med. Clin. N. Amer.* **34**, 1335–1362.